DIFFERING RATES OF DEATH AT INNER AND OUTER SURFACES OF THE PROTOPLASM

I. Effects of Formaldehyde on Nitella

By W. J. V. OSTERHOUT

(From the Laboratories of The Rockefeller Institute for Medical Research)

(Received for publication, March 15, 1944)

When protoplasm dies it becomes completely and irreversibly permeable and this may be used as a criterion of death.

On this basis we may say that in *Nitella* where the protoplasm forms a thin layer surrounding the cell sap, death in formaldehyde may occur sooner at the inner than at the outer protoplasmic surface.

The outer and inner surfaces are non-aqueous layers which may be called respectively X and Y. Between these is an aqueous layer, W, which makes up the bulk of the protoplasm. Outside X is the cellulose wall which is so permeable that it may be neglected in the subsequent discussion.

The non-aqueous layers X and Y are the seats of the electrical potentials developed in the cell. When we apply formaldehyde we can tell which layer is being altered by following the changes in potential.

The potentials across X and Y are due to concentration gradients of electrolytes, chiefly of KCl.[1] The sap contains about 0.05 M KCl which appears to be much greater than the concentration in W. As a result there is a positive[2] diffusion potential of about 100 mv. across Y: this will be designated as P_y. The potential across X (P_x) may be positive, negative, or zero. When the cell is bathed in tap water or in 0.00001 M KCl, P_x appears to be very small.[3]

It is convenient to have a designation for the total potential ($P_x + P_y$) with a fixed external solution and we may therefore call the potential across the protoplasm when the outside solution is 0.001 M KCl the "standard potential."

To measure the potential[4] the cell was placed on a paraffin block and solutions

[1] The influence of KCl on potential is predominant so that for convenience the effects of other salts may be neglected in this discussion.

[2] The potential is called positive when the positive current tends to flow from the sap across the protoplasm to the external solution.

[3] When the external solution is tap water or 0.00001 M KCl and X is made insensitive to K^+ so that P_x disappears the total potential across the protoplasm does not fall off. *Cf.* Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933–34, **17**, 87.

[4] The cells, after being freed from neighboring cells, stood in the laboratory at 15°C. ± 1°C. in Solution A (*cf.* Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*,

were applied at three spots designated as *A*, *B*, and *C*. Under each of these spots there was an excavation in the paraffin block which held several milliliters of solution. Between each of these spots there was an excavation (2 cm. wide) filled with air: this sufficed to prevent short-circuiting. *A* and *B* were connected through a recording Einthoven galvanometer (with amplifier) to *C* which was in contact with 0.01 M KCl which kept the potential constant, approximately at zero.[5]

Effects of Formaldehyde on the Inner Protoplasmic Surface, Y.—When we apply 0.001 M KCl plus 0.2 M formaldehyde[6] the potential falls off and eventually disappears. Since *X* has little or no potential at the start the loss of potential must occur at *Y* and since it is irreversible we may conclude that *Y* becomes completely permeable.

When formaldehyde is applied there is at first a period[7] during which the curve falls a little (Fig. 1) or remains constant: this varies between 30 seconds and 2 minutes. After this the rise of the curve may be gradual throughout, as in Fig. 1, or it may begin in this way and suddenly rise.[8] The time required for the curve to reach zero[9] varies between 6 and 60 minutes.

1933–34, **17,** 87) for several days. Fig. 1 refers to cells in Lot B, Fig. 2 to cells in Lot A (*cf.* Hill, S. E., and Osterhout, W. J. V., *Proc. Nat. Acad. Sc.*, 1938, **24,** 312).

The measurements were made on *Nitella flexilis*, Ag., using the technique described in former papers (Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1937–38, **21,** 541). Temperature 20–26°C. Regarding the amplifier see the reference just cited.

[5] Two spots on the cell, *A* and *B*, were connected to a spot *C* through a recording galvanometer. At the end of the experiment *A*, *B*, and *C* were killed (in this order) by applying chloroform, which reduced the P.D. at each spot to zero.

It was then possible to ascertain the potential across the protoplasm at *A* and *B* at any previous point on the record on the assumption that *C* had remained constant up to that point (or by correcting for any change). If *C* had changed the amount of alteration could be detected because it would appear as a simultaneous change at *A* and *B* (in the same direction at both).

[6] This does not plasmolyze the cells used in these experiments. When the cells are dying we may see what has been called false plasmolysis. *Cf.* Osterhout, W. J. V., *Bot. Gaz.*, 1913, **55,** 446.

[7] This appears to be due to a change in *X*. Some time is needed for penetration to *Y* (and the consequent rise of the curve) and this may be regarded as a latent period in respect to *Y*. Regarding latent periods in *Nitella* and other large plant cells see Osterhout, W. J. V., *J. Gen. Physiol.*, 1936–37, **20,** 13; 1939–40, **23,** 569; 1940–41, **24,** 311, 699; *J. Cell. and Comp. Physiol.*, 1941, **18,** 129. Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1938–39, **22,** 107.

[8] The sudden rise of the curve will be discussed in a subsequent paper.

[9] The time depends somewhat on the area of the cell covered by the solution. In these experiments about one-third of the cell was covered.

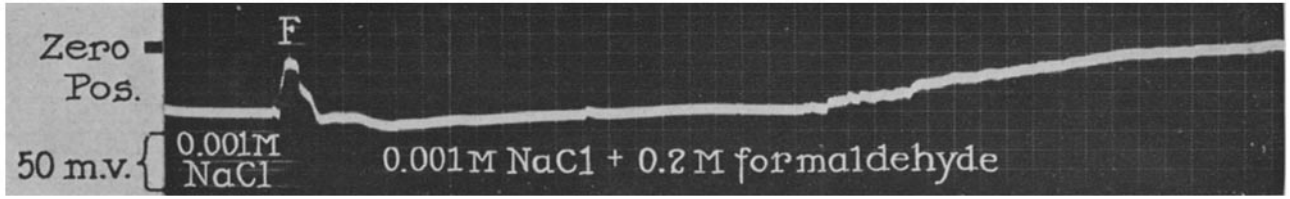

FIG. 1. The rise of the curve to zero shows the loss of potential under the influence of formaldehyde. At first the recorded region of the cell was in contact with 0.001 M NaCl. When the solution was removed this region was no longer in the circuit and the curve jumped to the "free grid" level marked "*F*." Then 0.001 M NaCl + 0.2 M formaldehyde was applied and the curve went back approximately to the previous level and after a slight dip began to rise.

The recorded spot was connected through a recording galvanometer to another spot in contact with 0.01 M KCl which kept the P.D. constant approximately at zero.

The cell was freed from neighboring cells and kept in Solution A for 29 days at 15°C. ± 1°C. and then placed in 0.001 M NaCl for 1 hour at 25°C. before the experiment was performed.

Time marks 15 seconds apart.

When the curve has risen to zero it usually remains there unless the external electrolyte is changed. But in some cases there is a temporary fall of the curve amounting to a few millivolts which may be explained on the ground that as Y becomes permeable (as shown by the previous loss of potential) KCl comes out of the sap and reaches X, thus setting up some potential which disappears[10] as KCl diffuses out through X.

The gradual rise in the curve in Fig. 1 indicates an increase in permeability of Y to electrolytes and especially to KCl and a loss of its potential[11] as KCl moves from the sap to the outside of Y (*i.e.*, into W). When the outwardly moving KCl reaches X it can set up a noticeable positive potential if it advances with a sharp diffusion boundary and a sufficient concentration gradient but not if it enters W slowly and diffuses out through X as appears to be the case here.

Effects of Formaldehyde on Ionic Mobilities in X.—After Y becomes completely permeable, as shown by the total loss of standard potential, the outer protoplasmic surface, X, is still capable of responding to changes in electrolytes. This is shown by measurements of the concentration effect at X.[12] For example, if X has been standing for some time in contact with 0.001 M KCl which penetrates into W and this is replaced by 0.01 M KCl which begins to penetrate X we may have the situation shown in Scheme 1[13] (any possible phase boundaries are neglected).

	Aqueous 0.01 M KCl
X	Layer in approximate equilibrium with 0.01 M KCl
	- - - - - - - - - - - - Diffusion boundary
	Layer in approximate equilibrium with 0.001 M KCl

SCHEME 1

[10] This is analogous to what frequently happens in the action current. *Cf.* Osterhout, W. J. V., *J. Gen. Physiol.*, 1934–35, **18,** 215.

[11] In some cases the curve goes above zero while in contact with formaldehyde or just after its removal, indicating a negative potential at X.

[12] In this the immediate rise of the curve after applying the new solution shows that the effect is at X, since there is not sufficient time for the new solution to penetrate to Y.

[13] If a diffusion boundary and a diffusion potential exist in X the application of a suitable external solution will cause a change in potential due to the new diffusion boundary set up outside the old one. We may use the equation, putting the P.D. across the new diffusion boundary equal to the change of potential.

This gives a diffusion potential[14] of 28 mv. Previous experiments indicate[15] that in such cases we may calculate ionic mobilities by means of the usual equation which may be written (for 25°C.)

$$\text{P.D.} = 59 \frac{u_K - v_{Cl}}{u_K + v_{Cl}} \log \frac{a_1}{a_2}$$

where u_K and v_{Cl} are the mobilities of K^+ and Cl^- in X and $a_1 \div a_2$ is the ratio of activities of KCl in the outer and inner regions of X. Previous experiments[16] indicate that we may regard this as approximately equal to the ratio of concentrations in the external aqueous solutions; *i.e.*, as 0.01 ÷ 0.001.

TABLE I

Potential Differences, Relative Ionic Mobilities, and Partition Coefficients before and after Exposure for 7 Minutes to 0.001 M NaCl + 0.2 M Formaldehyde at about 25°C.

Potassium effect 0.01 M KCl *vs.* 0.01 M NaCl		K concentration effect 0.01 M KCl *vs.* 0.001 M KCl		Na concentration effect 0.01 M NaCl *vs.* 0.001 M NaCl		Ratio of partition coefficients $S_{KCl} \div S_{NaCl}$		u_K = mobility of K^+ in X		u_{Na} = mobility of Na^+ in X	
Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
mv.	*mv.*	*mv.*	*mv.*	*mv.*	*mv.*						
33	0	28	20	33	25	17	1.4	2.81	2.03	3.54	2.47

For convenience we may put $v_{Cl} = 1$ and we then have, before treatment with formaldehyde (Table I),

$$\text{P.D.} = 28 = 59 \frac{u_K - v_{Cl}}{u_K + v_{Cl}} \log \frac{0.01}{0.001}$$

whence

$$u_K = 2.81$$

This means that $u_K \div v_{Cl} = 2.81$.

Proceeding in the same way we find for the concentration effect of NaCl, *i.e.* 0.01 *vs.* 0.001 M NaCl a P. D. of 33 mv. whence $u_{Na} \div v_{Cl} = 3.54$.

After treatment with formaldehyde[17] we find for the concentration effect

[14] This potential is set up within a few seconds after the change in solution; *i.e.*, before there is time for the new solution to penetrate to Y.

[15] Osterhout, W. J. V., *J. Gen. Physiol.*, 1929–30, **13,** 715.

[16] Osterhout, W. J. V., *J. Gen. Physiol.*, 1942–43, **26,** 293.

[17] In this connection we may recall the fact that other organic substances can change mobilities in X. *Cf.* Osterhout, W. J. V., *J. Gen. Physiol.*, 1536–37, **20,** 13, 685; 1937–38, **21,** 707; 1939–40, **23,** 171.

of KCl 20 mv. (Table I), whence $u_K \div v_{Cl} = 2.03$. For the concentration effect of NaCl we have 25 mv., whence $u_{Na} \div v_{Cl} = 2.47$.

This shows that X has not become completely permeable for in that case u_K would be practically equal to v_{Cl} (as in water) and the concentration effect would be nearly zero.[18] We find, however, that good concentration effects at X may be obtained more than an hour after Y has become completely permeable (as shown by the complete loss of the standard potential across the protoplasm). We may, therefore, conclude that death arrives sooner at Y than at X.

Effect of Formaldehyde on Partition Coefficients As Related to the Potassium Effect.—Under normal conditions X is able to distinguish electrically between Na^+ and K^+. For example, when the cell has stood for some time in contact with 0.01 M KCl and this is replaced by 0.01 M NaCl a diffusion boundary is set up in X between KCl and NaCl. In consequence there is a diffusion potential which (Table I, p. 27) before treatment with formaldehyde amounts to 33 mv.

In previous papers [19] it has been customary to calculate partition coefficients by means of Henderson's equation which may be written for 25°C.

$$\text{P.D.} = 59 \frac{(U_I - V_I) - (U_{II} - V_{II})}{(U_I + V_I) - (U_{II} + V_{II})} \log \frac{U_I + V_I}{U_{II} + V_{II}}$$

where $U_I = u_K c_K$, $V_I = v_{Cl} c_K$, $U_{II} = u_{Na} c_{Na}$, $V_{II} = v_{Cl} c_{Na}$. For convenience we put $v_{Cl} = 1$ and $c_{Na} = 1$ and insert the values already found (see Table I, p. 27); *i.e.*, $u_K = 2.81$ and $u_{Na} = 3.54$. We then find if we put $c_{Na} = 1$ and $c_K = 17$ that the calculated P.D. is 33 which is the observed value. This means that if we define the partition coefficient S as the concentration in X divided by the concentration in the external solution we may put $S_{KCl} \div S_{NaCl} = 17$ since the concentrations of KCl and NaCl in the external solutions are equal.

The treatment with formaldehyde lowers the value of the potassium effect[20] and may eventually reduce it to zero.[21] This is accompanied by a change in the ratio of partition coefficients. To calculate this change we may use the values for mobilities found after treatment with formaldehyde; *i.e.*, $u_K =$ 1

[18] In water the concentration effect of NaCl would be greater than that of KC but in the freshly killed cell there is little difference because the sap comes out at once and as this contains 0.05 M KCl it cuts down the diffusion potential of NaCl.

[19] *Cf.* Hill, S. E., and Osterhout, W. J. V., *Proc. Nat. Acad. Sc.*, 1938, **24,** 312. Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1938–39, **22,** 139.

[20] The potassium effect may be completely abolished by an exposure for 1 minute to 0.1 M KCl + 0.2 M formaldehyde without making X completely permeable.

[21] After the loss of the potassium effect the concentration effects may persist for an hour or more.

2.03 and $u_{Na} = 2.47$ (Table I, p. 27). We then find that if we put $c_K = 1.4$ and $c_{Na} = 1$ we obtain for the calculated P.D. a value of zero, as observed. This gives $S_{KCl} \div S_{NaCl} = 1.4$ instead of 17, the value found before treatment.

Other cases where organic substances change partition coefficients have been observed in previous experiments with living cells[22] and with models.[23] This deserves further study.

Restoration of the Potassium Effect.—The potassium effect can also be removed by treatment with distilled water,[24] with acid, [25] and with alkali, and may be restored by the application of blood. An attempt was therefore made to restore the potassium effect after it had been removed by 0.1 M KCl + 0.2 M formaldehyde but the restoration was incomplete.[26, 27]

When the potassium effect is removed by distilled water the restoration by blood is usually complete.[28] This has been explained on the ground that the potassium effect depends on a substance (or group of substances) called for convenience R which is removed by distilled water and restored by the application of blood. When the restoration is incomplete, as after treatment with formaldehyde, it would seem that X has been altered so that even in the presence of R it cannot produce the complete potassium effect.

This throws an interesting light on variations in the potassium effect under normal conditions for it seems possible that substances are produced in metabolism which condition X so that even in the presence of a normal amount of R variations in the potassium effect occur. The values of the potassium effect under normal conditions range[29] from 26 mv. to 95 mv. Likewise the concentration effect of KCl and of NaCl varies and may depend on conditioning substances. The value for KCl (0.01 M *vs.* 0.001 M) ranges [30] from 22 mv. to 54 mv. The value for NaCl ranges from 20.9 mv.[15] to 34 mv.[31]

In the same way when a normal amount of R is present conditioning sub-

[22] *Cf.* Osterhout, W. J. V., *J. Gen. Physiol.*, 1939–40, **23,** 171, 749.

[23] *Cf.* Osterhout, W. J. V., *J. Gen. Physiol.*, 1943–44, **27,** 91.

[24] Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933–34, **17,** 87, 105.

[25] Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933–34, **17,** 99.

[26] For example, if the potassium effect before treatment is 40 mv. and this disappears during exposure to formaldehyde the subsequent application of blood for 1 minute may restore it to 15 mv.

[27] Citrated whole sheep's blood was dried at 50°C. and an aqueous extract was made which had the same concentration as fresh blood diluted with 4 parts of water.

[28] Osterhout, W. J. V., *J. Gen. Physiol.*, 1935–36, **19,** 423.

[29] Osterhout, W. J. V., *J. Gen. Physiol.*, 1929–30, **13,** 715; 1938–39, **22,** 417; 1939–40, **23,** 171; 569.

[30] Osterhout, W. J. V., *J. Gen. Physiol.*, 1929–30, **13,** 715; 1934–35, **18,** 987. Osterhout, W. J. V., and Hill, S. E., *Proc. Nat. Acad. Sc.*, 1938, **24,** 427.

[31] Unpublished results. See also footnote 30.

stances may influence the potential across Y. This appears to vary from 60 mv. to 200 mv.[32]

Effects of Formaldehyde on Turgidity.—The sap contains about 0.1 M chlorides plus some other substances which give an excess of osmotic pressure over the external solutions used in these experiments. As a result water enters the vacuole and produces a hydrostatic pressure which forces the protoplasm against the cellulose wall as an inner tube is forced against the outer casing of an automobile tire. The cell is then said to be in a turgid state. The pressure amounts to about 4 atmospheres.[33]

As a result a cell less than 1 mm. in diameter and 100 mm. long acquires so much mechanical rigidity that when grasped at one end and held in a horizontal position it does not bend perceptibly under it own weight. When the cell dies it becomes as flabby as a wet thread.

In order to test the effects of formaldehyde on the turgidity (*i.e.* rigidity) of the cell it was placed in a horizontal position over an excavation 2 cm. wide in the paraffin block. Thus the cell was supported only at the edges of the excavation. A ring of platinum wire weighing 0.175 gm. was suspended from the cell in the middle of the excavation which was filled with solution. As long as the cell retained its normal turgidity this weight did not cause the cell to sag perceptibly in the middle of the excavation but as soon as the turgidity fell off it began to do so.

When this excavation was filled with a solution of formaldehyde (which extended further along the cell so as to cover about one-third of its surface) the turgidity did not fall off although the potential had disappeared, indicating that Y had become completely permeable. It would seem, however, that X had not become completely permeable and it retained some of the osmotically active substances (not necessarily electrolytes effecting P.D.) so that turgidity was maintained. If X became permeable to these substances we should expect the turgidity to disappear. It can be made permeable by applying 0.001 M NaCl saturated with chloroform and when this was done the turgidity disappeared.

Unlikeness of the Inner and Outer Protoplasmic Surfaces.—It may seem surprising that the reagent which must pass through X in order to reach Y should produce death at Y sooner than at X. We know, however, from previous experiments that X and Y differ. Even when both are in contact with the same solution they behave differently. For example, when we set up the chain

Sap | Protoplasm | Sap

[32] Unpublished results. These values are found when the external solution is 0.001 M NaCl.

[33] The concentration of chlorides in the sap is about 0.1 M (about 0.05 M KCl + 0.05 M NaCl) which is osmotically equivalent to about 0.2 M cane sugar solution or to about 0.2(22) = 4.4 atmospheres. Hence it requires about 0.25 M cane sugar to plasmolyze. Smaller cells have less osmotic pressure than larger cells.

we obtain a value of 15 mv. which would be impossible if X and Y were identical in properties.[34]

It is, therefore, not surprising that differences are found when X and Y are in contact with unlike solutions.

We find differences in respect to the penetration of NaCl and other salts.[35] For example, when large cells are placed in 0.3 M NaCl the salt penetrates X more rapidly than Y and in consequence the osmotic pressure rises and W absorbs water from the vacuole.

We also find that certain organic substances can change the properties of X without making much change in the standard potential so that evidently there is not much change in Y.[22]

In the experiments with formaldehyde plus 0.001 M NaCl the potential across Y is very much greater than across X owing to the greater concentration gradient of KCl across Y and in consequence the electrical pressure across Y is much greater. If the potential across Y is 100 mv. and the thickness of Y is 0.1 micron the electrical pressure is 1000 volts per mm. It may be even greater since Y may be less than 0.1 micron in thickness.

It is not surprising that the increase in permeability should be linked to electrical pressure, since an increase in electrical pressure of 100 to 500 mv. can produce in *Nitella* the increase in permeability which leads to the action current. It must be remembered that under normal conditions the electrical pressure at any spot is approximately balanced by that at other spots so that little or no current flows.

It is also quite possible that the concentration of KCl may influence the result quite apart from its effect on electrical pressure.

In view of this it is desirable to test the effects of formaldehyde when X and Y are in contact with similar solutions. Since KCl plays an important rôle it was thought desirable to pay especial attention to it. As the sap contains about 0.05 M KCl a solution of 0.01 M KCl + 0.17 M formaldehyde was applied to X. The result[36] is shown in Fig. 2. Here it would appear that X loses its potential and becomes permeable more rapidly than Y[37] because the reagent reaches X first.

The curve shows the total potential across the protoplasm. At the start the external solution was 0.01 M KCl and the potential was approximately zero (Fig. 3). Then 0.01 M KCl + 0.17 M formaldehyde was applied and as X

[34] Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1927–28, **11,** 391.

[35] Osterhout, W. J. V., *J. Gen. Physiol.*, 1943–44, **27,** 139.

[36] The cells used in these experiments were from a different lot than that employed in the other experiments described in this paper.

[37] *Cf.* Osterhout, W. J. V., Some aspects of permeability and bioelectrical phenomena, in Molecular physics in relation to biology, *Bull. Nat. Research Council, No.* **69**, 1929, 170. Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1928–29, **12,** 355.

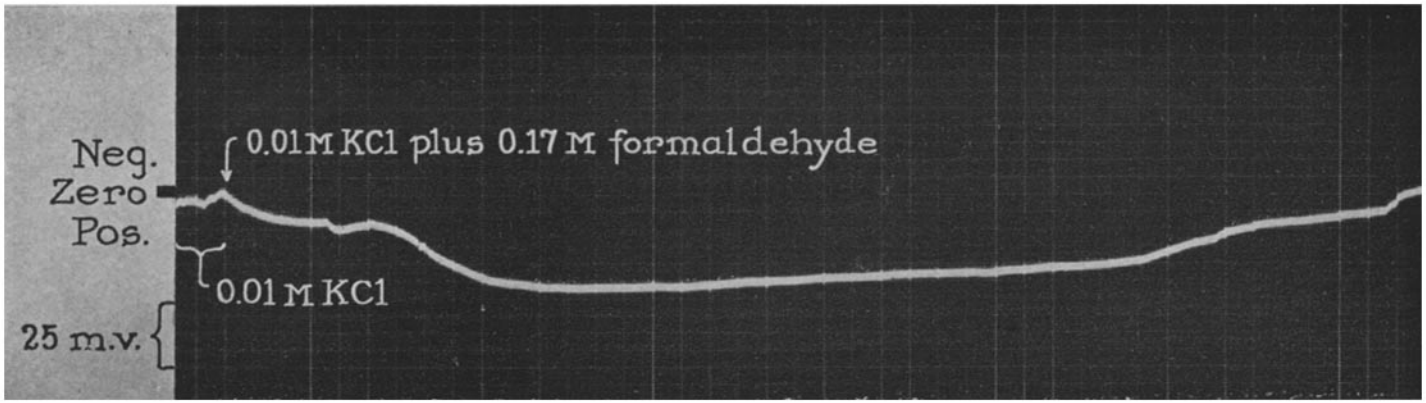

FIG. 2. The fall of the curve shows the increase of permeability of the outer non-aqueous protoplasmic layer X. The subsequent rise of the curve shows the increase of permeability of the inner non-aqueous protoplasmic layer Y. The protoplasm was in contact with 0.01 M KCl + 0.17 M formaldehyde which gave X at the start a negative potential of about 100 mv. which will be called P_x. At Y there was a positive potential of about the same value: this will be called P_y. The total potential, $P_x + P_y$, was equal to zero. *Cf.* Fig. 3.

Under the influence of formaldehyde the outer protoplasmic surface X became more permeable and P_x diminished so that the curve fell. Later the inner protoplasmic surface Y became more permeable and P_y diminished so that the curve rose.

The recorded spot was connected through a recording galvanometer to a spot killed by chloroform in 0.01 M KCl which consequently had a P.D. value of zero.

The cell was freed from neighboring cells and kept in Solution A for 3 days at 15°C. ± 1°C. It was then kept for 1 hour at about 22°C. before the experiment was made. Time marks 5 seconds apart.

lost its potential more rapidly than Y the total potential became more positive and the curve fell. After about 1 minute the loss of potential at Y became faster than at X so that the total potential decreased and the curve began to rise.[38–41]

We may therefore conclude that death may occur at different rates in different parts of the cell and that this can be brought under experimental control.

Potentials at X and Y.—When the cell is treated with formaldehyde we often find that soon after the curve has risen to zero the concentration effect of KCl and of NaCl at X is the same as before treatment. We may therefore conclude

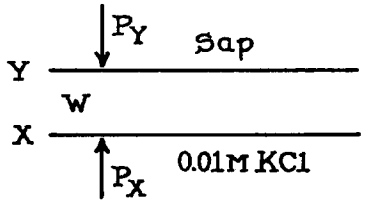

FIG. 3. Hypothetical diagram to illustrate potentials at the start of the curve in Fig. 2. The positive potential at Y, *i.e.* P_y, is equal to the negative potential at X, *i.e.* P_x. When 0.01 M KCl plus 0.17 M formaldehyde is applied P_x begins to diminish, and the curve in Fig. 2 begins to fall. As formaldehyde penetrates to Y the value of P_y falls off and the curve in Fig. 2 begins to rise.

that there has been little or no change in the permeability of X or in its reactions to electrolytes.

This simplifies our picture of the behavior of P_x. It has been stated (p. 23) that with 0.00001 M KCl outside the total potential ($P_x + P_y$) does not fall off when P_x is made to disappear so that we may put $P_x = 0$ and $P_y = 100$ mv. If after treatment with formaldehyde we find that $P_x + P_y = 0$ it is probable

[38] *Cf.* Osterhout, W. J. V., Some aspects of permeability and bioelectrical phenomena, in Molecular physics in relation to biology, *Bull. Nat. Research Council, No.* **69**, 1929, 170. The behavior of the curve varies with different cells; in some cases the drop is slower and less pronounced.

[39] Similar effects are obtained with 0.1 M KCl + 0.01 M $HgCl_2$.

[40] This is quite the opposite of what happens when a reagent produces alteration at Y earlier than at X and the potential at Y falls after which KCl coming out and reaching X sets up a potential. In this case the potential first becomes more negative and then more positive. *Cf.* Osterhout, W. J. V., *J. Gen. Physiol.*, 1934–35, **18**, 215.

[41] If we apply 0.01 M KCl alone the curve remains for a long time at the same level and does not show the fall and rise seen in Fig. 2.

that the loss of potential and the change in permeability has been confined to Y.[42,43]

If the external solution is 0.00001 M KCl and $P_x = 0$ we may say that the concentration of KCl in W is 0.00001 M if we assume that the P.D. is wholly due to KCl.[44,45]

This picture of KCl with a low concentration externally and in W and a relatively high concentration in the sap corresponds to what is observed with such dyes as brilliant cresyl blue and neutral red. When the concentration of dye is very low in the external solution it is very low in W but may be relatively high in the sap.[46]

If the concentration effect of 0.001 M *vs.* 0.01 M KCl is 33 mv. (a value commonly found in this lot of cells) we may write

$$31 = 59 \frac{u_K - v_{Cl}}{u_K + v_{Cl}} \log \frac{0.01}{0.001}$$

whence[47] $u_K \div v_{Cl}$ in $X = 3.54$. Hence if we have 0.00001 M KCl in W and 0.01 M KCl outside X we may expect for the value of P_x (putting $v_{Cl} = 1$)

$$P_x = 59 \frac{3.54 - 1}{3.54 + 1} \log \frac{0.01}{0.00001}$$

$$= 99 \text{ mv.}$$

This will have a negative sign so that the total potential will be $P_y + (-P_x)$ $= 100 - 99 = 1$ mv. which is the observed value.

[42] This appears to be the case during stimulation. *Cf.* Osterhout, W. J. V., *J. Gen. Physiol.*, 1943–44, **27,** 61.

[43] Regarding a temporary increase in P_x in some cases see p. 26.

[44] This may be allowable as a first approximation.

[45] Brooks has reported relatively high concentrations of electrolytes in the protoplasm but these may be in organic combination and not in ionic form. *Cf.* Brooks, S. C., The intake of radioactive isotopes by living cells, in Cold Spring Harbor symposia on quantitative biology, Cold Spring Harbor, Long Island Biological Association, 1940, **8,** 173.

[46] Regarding brilliant cresyl blue see Irwin (Irwin, M., *J. Gen. Physiol.*, 1925–28, **8,** 147; 1925–26, **9,** 235, 561; 1926–27, **10,** 75, 927; 1927–28, **11,** 123; 1928–29, **12,** 147, 407; *Proc. Soc. Exp. Biol. and Med.*, 1926–27, **24,** 425; 1927–28, **25,** 127; 1928–29, **26,** 125, 135; 1931–32, **29,** 993, 995, 1234). The behavior of the dye depends on pH which does not seem to be the case with KCl (*cf.* Jacques, A. G., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1934–35, **18,** 967).

[47] A similar calculation for Y gives

$$100 = 59 \frac{u_K - v_{Cl}}{u_K + v_{Cl}} \log \frac{0.05}{0.00001}$$

whence $u_K \div v_{Cl}$ in $Y = 2.7$

When we place sap outside X we find as a rule that the total potential[34] is about 15 to 20 mv. negative so that $P_x = 115$ to 120 mv. negative when $P_y = 100$. This is to be expected since we may write (putting $u_K = 3.54$ and $v_{Cl} = 1$)

$$P_x = 59 \frac{3.54 - 1}{3.54 + 1} \log \frac{0.5}{0.00001}$$

$$= 122 \text{ mv.}$$

These values[48] are given merely by way of illustration: they may vary with each lot of cells.[49]

DISCUSSION

These results have a direct bearing on some fundamental questions. Death makes the protoplasm completely and permanently permeable so that this may be used as a criterion of death. On this basis we may say that death occurs sooner at the inner non-aqueous protoplasmic surface than at the outer. The inner surface is the chief seat of the positive potential and when this potential disappears we may regard the inner surface as completely permeable.

When the outer surface becomes completely permeable to electrolytes it can no longer distinguish electrically between different concentrations of the same salt. But this may not occur for an hour or more after the inner surface becomes completely permeable.

Before this, however, a change occurs in X which leads to the disappearance of the potassium effect. This change is due chiefly to alterations in the partition coefficients of KCl and NaCl in X. Before exposure to formaldehyde the ratio of partition coefficients (*i.e.*, $S_{KCl} \div S_{NaCl}$) is 17; after exposure it is 1.4 (Table I, p. 27). Hence the concentration of Na^+ in X approaches that of K^+ and the potassium effect falls off.

It is evident that the properties of the non-aqueous protoplasmic surfaces

[48] The relatively low concentration of KCl provisionally suggested for W is in line with experiments on basic dyes: no matter how great the concentration of dye in the sap the concentration in W is always very low. Brooks (Brooks, S. C., Cold Spring Harbor symposia on quantitative biology, Cold Spring Harbor, Long Island Biological Association, 1940, **8,** 171) has stated that relatively high concentrations of radioactive salts may occur in the protoplasm (as compared with the sap) but it seems possible that they may be in combination with organic substances: there is no proof that they are present in ionic form in the protoplasm at high concentrations.

[49] For higher values of the concentration effect of KCl see Osterhout, W. J. V., *J. Gen. Physiol.*, 1929–30, **13,** 715; Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1937–38, **21,** 541; *Proc. Nat. Acad. Sc.*, 1938, **24,** 312. These correspond to higher concentrations of KCl in W. For higher values of P_x see Osterhout, W. J. V., *J. Gen. Physiol.*, 1943–44, **27,** 61.

can be brought under experimental control to a considerable degree. This deserves further study.

SUMMARY

When protoplasm dies it becomes completely and irreversibly permeable and this may be used as a criterion of death. On this basis we may say that when 0.2 M formaldehyde plus 0.001 M NaCl is applied to *Nitella* death arrives sooner at the inner protoplasmic surface than at the outer.

If, however, we apply 0.17 M formaldehyde plus 0.01 M KCl death arrives sooner at the outer protoplasmic surface.

The difference appears to be due largely to the conditions at the two surfaces. With 0.2 M formaldehyde plus 0.001 M NaCl the inner surface is subject to a greater electrical pressure than the outer and is in contact with a higher concentration of KCl. In the other case these conditions are more nearly equal so that the layer first reached by the reagent is the first to become permeable.

The outer protoplasmic surface has the ability to distinguish electrically between K^+ and Na^+ (potassium effect). Under the influence of formaldehyde this ability is lost. This is chiefly due to a falling off in the partition coefficient of KCl in the outer protoplasmic surface.

At about the same time the inner protoplasmic surface becomes completely permeable. But the outer protoplasmic surface retains its ability to distinguish electrically between different concentrations of the same salt, showing that it has not become completely permeable.

After the potential has disappeared the turgidity (hydrostatic pressure inside the cell) persists for some time, probably because the outer protoplasmic surface has not become completely permeable.